



Monitoring Expansion of T Cell Specificities Against Foot-and-Mpouth-Disease Virus (FMDV) in Swine With MHC Class I Tetramers Following a Prime/Boost Vaccination

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recognised the M, N and G proteins. BoLA A14 cattle recognised the G, F, M and M2 proteins; and BoLA A10 cattle recognised the G, M, N and NS1 proteins of BRSV. MHC class I-restriction of IFN γ production was demonstrated using BRSV-infected P815 cells expressing individual bovine MHC class I genes. In addition, CD8+ T cells restimulated in vitro with rFPV expressing BRSV proteins showed MHC-restricted lysis of BRSV-infected skin fibroblasts. Using overlapping peptides from the BRSV P protein, a BoLA 6*0301-restricted epitope was identified and used to produce tetramers, which stained P protein-specific MHC-matched, but not MHC-mismatched, CD8+ T cells. Following restimulation with BRSV, in vitro, a proportion of CD8+ T cells were identified that also expressed CD4. The proportion of CD8+CD4+ T cells increased with successive restimulation, in vitro. These double positive cells were BRSV protein-specific and CD3+, CD8 α / β +, NKp46-, γ / δ TCR-. Further studies are required to determine the role of these cells in BRSV infection, in vivo.

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MONITORING EXPANSION OF T CELL SPECIFICITIES AGAINST FOOT-AND-MOUTH-DISEASE VIRUS (FMDV) IN SWINE WITH MHC CLASS I TETRAMERS FOLLOWING A PRIME/BOOST VACCINATION

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This study evaluates the expansion of cytotoxic T cell (CTL) specificities raised against foot-and-mouth-disease virus (FMDV) as a result of vaccination and boost. Using two recombinant swine MHC class I molecules, the SLA-1*0401 and SLA-2*0401, a total of 15 different SLA "FMDV-tetramers" were generated with peptides derived from the P1 capsid region of the FMDV A24 strain. Four SLA matched animals were immunized using a combination of B- and T- cell directed vaccines based on an Adeno virus (Ad5) vectored platform containing either an intact or a mutated 3Cpro gene sequence of the FMDV A24 strain, respectively. To enumerate peptide-specific CTL responses, all tetramers were analyzed for their ability to stain CD6+/CD4-/CD8+ porcine PBMCs. These analyses revealed two dominant FMDV-tetramer specificities post priming and first boost. Interestingly, an expansion of reactive CTL types were observed after a second boost, seeing multiple CTL specificities staining positive with different FMDV-tetramers of which four were clearly dominant in all animals. These results suggest an immune response against FMDV established as a result of prime and boost not only remains, but actually expands following a second boost, thereby drawing out T cell clones of lower precursor frequency to contribute to the adaptive immune response. Finally, these techniques demonstrate promising potential for analysis of vaccine performance in the development of porcine vaccines to intracellular pathogens, particularly viruses.